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Reduction of the hydraulic retention time at constant high organic loading rate to reach the microbial limits of anaerobic digestion in various reactor systems

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HIGHLIGHTS

- Anaerobic digestion at extreme short HRT was investigated in various reactors.
- Functional markers for H_2 and CH_4 producing pathways were analyzed on mRNA level.
- Stable isotope analysis of the biogas was applied to assess methanogenic pathways.
- Depending on HRT *Clostridiales* and *Spirochaetales* were the most active in reactors.
- Increased activity of *Methanosaeta* was found during HRT decrease in CSTR and ASBR.

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ABSTRACT

The effects of hydraulic retention time (HRT) reduction at constant high organic loading rate on the activity of hydrogen-producing bacteria and methanogens were investigated in reactors digesting thin stillage. Stable isotope fingerprinting was additionally applied to assess methanogenic pathways. Based on *hydA* gene transcripts, *Clostridiales* was the most active hydrogen-producing order in continuous stirred tank reactor (CSTR), fixed-bed reactor (FBR) and anaerobic sequencing batch reactor (ASBR), but shorter HRT stimulated the activity of *Spirochaetales*. Further decreasing HRT diminished *Spirochaetales* activity in systems with biomass retention. Based on *mcrA* gene transcripts, *Methanoculleus* and *Methanosarcina* were the predominantly active in CSTR and ASBR, whereas *Methanosaeta* and *Methanospirillum* activity was more significant in stably performing FBR. Isotope values indicated the predominance of aceticlastic pathway in FBR. Interestingly, an increased activity of *Methanosaeta* was observed during shortening HRT in CSTR and ASBR despite high organic acids concentrations, what was supported by stable isotope data.

1. Introduction

One of the rapidly advancing areas of modern biotechnology is the development of sustainable waste treatment technologies. Much attention is paid to the efficient anaerobic digestion of organic substances, including agricultural, industrial, and household wastes, with the production and utilization of energy rich biogas. Anaerobic conversion of waste organic matter into biogas in engineered systems is also a promising way of reducing uncontrolled greenhouse gas emissions. However, it is also recommended to improve the digestate storage to reduce further methane emissions (Mata-Alvarez et al., 2000; Holm-Nielsen et al., 2009).

Among the main operational conditions that need to be periodically monitored in anaerobic digesters, hydraulic retention time (HRT) is one of the key process parameters. Assuming volume constancy this parameter is calculated from the working volume of the reactor that is divided by the daily feeding volume of the substrate; therefore, it is closely linked to the organic loading rate (OLR) and defines the average residence time of the substrate in the system. Current biogas plants in the agricultural sector usually apply long

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